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14. ABSTRACT

Radiotherapy is a common therapeutic modality for the treatment of human prostate cancer. However, tumors often demonstrate resistance to ionizing radiation and continue to proliferate under genotoxic stress. The goal of this project is to determine whether silencing of MEK5 will sensitize prostate cancer cells to ionizing radiation. Mitogen-activated protein kinase kinase 5 (MAP2K5/MEK5) is a member of the MAPK family of protein kinases. MEK5 is often overexpressed in human prostate tumors and it is involved in tumor initiation and progression. In the current funding period we have been able to show that treatment of prostate cancer cells with ionizing radiation leads to activation of ERK5, which is the sole downstream effector of MEK5, whereas depletion of MEK5 sensitizes prostate cancer cells to γ-radiation as determined by both clonogenic survival and cell proliferation assays. Furthermore, MEK5 silencing impacts on the activation of key modulators of the DNA damage response pathway. In addition, Akt activation in response to ionizing radiation is reduced by MEK5 downregulation. Altogether, these findings suggest that MEK5 could be targeted for therapeutic purposes in prostate cancer patients treated with ionizing radiation.

15. SUBJECT TERMS
MEK5, Akt, DNA damage response, ionizing radiation, prostate cancer

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INTRODUCTION

Radiation is the most critical therapeutic modality for treating human prostate cancer. However, tumor recurrence and therapy resistance often ensue. Moreover, radiation is not free of other serious unwanted risks, including the promotion of secondary cancer (1), as well as increased tumor-associated angiogenesis and metastasis, especially when cells are subjected to sublethal doses of radiation. A major cause of failure in radiation treatment is intrinsic and therapy-induced radioresistant tumor cells. The major mechanisms for radiotherapeutic resistance are the DNA damage and repair pathway, as well as cell membrane-associated prosurvival pathways such as EGF receptor and phosphoinositide 3-kinase (PI3K)/Akt. Although the delivery of higher doses of ionizing radiation improves local control (2-4), there are constraints due to dose-limiting toxicities to noncancerous tissues (5-7). Thus, lowering radiation dose, while preserving therapeutic index, is a goal in both the laboratory research setting and the clinic.

Mitogen-activated protein kinase kinase 5 (MAP2K5, MEK5, or MKK5), belongs to the family of MAP kinases. It is activated by the upstream kinases MEKK2 and MEKK3 at S311/T315, or in some cases directly by c-Src (8-11). MEK5, in turn, phosphorylates and activates extracellular signal-regulated kinase 5 (ERK5, big-MAPK1, or BMK1) at T218/Y220 (8). ERK5 constitutes a unique class of MAPKs that can modify the activity of targets not only as a kinase through phosphorylation, but also via physical interactions by acting as a transcriptional cofactor in a nonkinase manner (12). The MEK5/ERK5 pathway can be activated by various stimuli such as oxidative stress, growth factors, and mitogens downstream of receptor tyrosine kinases, and G protein-coupled receptors, and culminates in the activation of a large number of transcription factors, including MEF2 (myocyte enhancer factor 2), c-JUN, NF-κB, CREB, and transcription factors that control the epithelial-mesenchymal transition (EMT) program (13-18). Moreover, gene expression analysis has shown that the MEK5/ERK5 pathway may also control hypoxia-responsive genes by a mechanism independent of HIF-1α expression control, under normoxic conditions (19).

MEK5/ERK5 pathway plays a pivotal role in tumor initiation and progression, including prostate cancer. MEK5 protein is overexpressed in prostate cancer cells compared with normal cells and MEK5 levels are correlated with prostate cancer metastasis (20). Furthermore, high expression of ERK5 in prostate cancer has also been found to correlate with poor disease-specific survival and could serve as an independent prognostic factor (21) and ERK5 expression in prostate cancer is associated with an invasive phenotype (22),

This study demonstrates that MEK5 downregulation enhances radiosensitization in human prostate cancer cells. MEK5 silencing modulates the activation of ATM and CHK1/2 kinases, major players of the DNA damage response and repair pathway, as well as effectively inhibits activation of Akt in response to ionizing radiation.

KEYWORDS

MEK5, Akt, DNA damage response, ionizing radiation, prostate cancer

ACCOMPLISHMENTS

What were the major goals of the project?

Specific Aim 1
Task 1: Prepare cell lines stably expressing shMEK5, HA-MEK5, HA-MEK5DD (constitutively active kinase).
Task 2: Examine the effect of MEK5 downregulation on cell cycle progression, apoptosis, and DNA damage repair activation after treatment with ionizing radiation. Predictably induce radioresistance by ectopically expressing MEK5 in relatively radiosensitive prostate cell lines.
Cell lines to be used: DU145, PC3, PrEC [ATCC]; EP156T.

Specific Aim 2
Task 1: Evaluate the contribution of Akt to MEK5-induced radioresistance by employing both an Akt-specific inhibitor and Akt specific siRNAs in MEK5-expressing cells and expressing an active Akt construct in prostate cancer cells with reduced MEK5 levels: Measure the effect on cell radioresistance by clonogenic survival. Cell lines to be used: DU145, PC3 [ATCC].

What was accomplished under these goals?

(1) Prepared stable clones of MEK5 knockdown in PC3 cells.
(2) Demonstrated that MEK5 depletion sensitizes DU145 and PC3 cells to ionizing radiation by both clonogenic survival assays, as well as cell proliferation assays.
(3) Discovered that MEK5 knockdown differentially regulates activation of ATM and CHK2 kinases, major players in the DNA damage response pathway, response to ionizing radiation.
(4) Demonstrated that MEK5 is required for Akt activation in response to ionizing radiation.

Specific Aim 1
Task 1: Prepare cell lines stably expressing shMEK5, HA-MEK5, HA-MEK5DD (constitutively active kinase).

We have prepared stable cell lines of DU145 and PC3 expressing either a MEK5-specific shRNA (Sigma) or a control (scrambled) shRNA (Addgene). Lentiviral particles carrying the scrambled shRNA were generated in 293T cells using pLKO.1-Scrambled (shControl) along with the packaging vectors psPAX2 and pMD2.G. Lentiviral particles with pLKO.1-shMEK5 were purchased from Sigma and used to infect DU145 and PC3. After puromycin selection (1 μg/ml), we isolated several stable clones expressing *shControl* in both cell lines, as well as two PC3 cell clones (#12, #22) expressing *shMEK5*. Knockdown in PC3/*shMEK5* cells is more than 90% (**Figure 1**). DU145 cells with *shMEK5* are currently under selection.

In preparation for the MEK5 overexpression experiments we have purchased from Addgene and tested a number of MEK5 and ERK5 constructs in 293T cells (**Figure 2**). In particular, we have obtained the retroviral vectors pBABEpuro-HA-MEK5 (Plasmid #53198) and pWZLblasti-HA-ERK5 (Plasmid #53175) (23), and the lentiviral vectors expressing constitutively active MEK5, namely myr-FLAG-MEK5-pcw107 (Plasmid #64620), MEK5 DD (S311D, T315D)-pcw107-V5 (Plasmid #64619), as well as the control Luciferase-pcw107 (Plasmid #64648) (24). Retroviral particles were used by transfecting the vector to PT67 packaging cells (Clontech), whereas lentiviral particles have been prepared in 293T, using the psPAX2 and pMD2.G packaging vectors, as described above.

Aim 1 is still in progress.

Task 2: Examine the effect of MEK5 downregulation on cell cycle progression, apoptosis, and DNA damage repair activation after treatment with ionizing radiation. Predictably induce radioresistance by ectopically expressing MEK5 in relatively radiosensitive prostate cell lines.
Cell lines to be used: DU145, PC3, PrEC [ATCC]; EP156T.

We determined the impact of ionizing radiation on MEK5/ERK5 pathway activation. DU145 cells were irradiated with a range of γ-ray doses (0-10 Gy) and 20 minutes later, cells were examined for ERK5 activation by immunoblotting. The dose response experiment showed that phosphorylation of ERK5 at T^{218}/Y^{220} and activation was increased in response to IR. ERK5 phosphorylation was increased two-fold by 4 Gy γ-rays (**Figure 3**). Thus, all subsequent experiments were performed at that radiation dose. Furthermore, a time course experiment using PC3 cells revealed that ERK5 activation by IR reached maximal levels within 5-15 min (**Figure 4**). As expected two independent siRNAs (#10, #78) targeting MEK5 reduced ERK5 phosphorylation to undetectable levels.

We then proceeded to examine the effect of MEK5 knockdown on the radiosensitization of DU145, and PC3 cell lines by clonogenic survival analysis. Cells were transiently transfected with *Luciferase* (control) or up to four non-overlapping *MEK5* siRNAs. Two days later, untransfected and transfected cells were plated, allowed to attach overnight and irradiated at 0, 2, 4, and 6 Gy for DU145 cells or 0, 1, 2, and 3 Gy for PC3 cells. Eleven days later, cells were stained with crystal violet and colonies with > 50 cells were counted. Results showed that MEK5 silencing sensitized both prostate cancer cell lines to the effects of ionizing radiation (**Figure 5**).

In addition to clonogenic survival assays, we employed short-term cell proliferation assays to determine the effect of IR on DU145 and PC3 cell proliferation. We found that MEK5 silencing has a significant impact on cell proliferation. Cell proliferation of DU145 cells was diminished by more than 70% in the combination of MEK5 knockdown and 4 Gy γ-rays. In contrast, MEK5 depletion or IR administered separately reduced cell proliferation by about 20% and 30%, respectively (**Figure 6A**). Similar results were obtained with PC3, although PC3 were more sensitive to IR treatment (approximately 60% compared with control, unirradiated cells) (**Figure 6A**). The reduction in cell proliferation between cells exposed to IR alone and IR combined with MEK5 depletion was highly significant in both cell lines ($p < 0.005$ for DU145 cells; $p = 0.004$ for PC3). Finally, a dose response cell proliferation assay using both DU145 and PC3 cells corroborated the results (**Figure 6B**).

We have examined the cell cycle distribution of both PC3 and DU145 cells in response to MEK5 knockdown, IR, and a combination of IR with MEK5 depletion, 6, 9, or 24 hours post-IR. However, no major differences were detected in the cell cycle profile of these cells. Furthermore, we determined whether apoptotic cell death was induced in response to MEK5 depletion combined with IR, by analyzing caspase-3 cleavage and activation, as well as PARP-1 cleavage in DU145 and PC3 cells. However, no apoptosis induction was detected for up to 24 h post-irradiation. Trypan blue assay showed that DU145 cell death was increased modestly from 1.25% to 5.2% in irradiated, MEK5 knockdown cells, after 24 h. These data indicate that the MEK5-mediated radiosensitization of DU145 and PC3 cells does not involve significantly enhanced susceptibility to apoptosis.

The DNA damage response and repair (DDR) signaling network is activated in response to genotoxic stress, including IR. We determined the impact of MEK5 depletion combined with IR on a number of major players of the DDR pathway by analyzing their phosphorylation status. Treating DU145 or PC3 with 4 Gy γ-rays induced a robust DNA damage response as determined by increased phosphorylation of ATM at Ser^{1981}

and CHK2 at Thr^{68}, as well as CHK1 at Ser^{345}. A time course experiment using irradiated DU145 or PC3 cells, expressing normal levels of MEK5, showed that CHK2 phosphorylation at Thr^{68} was increased 15 -30 min post-IR, diminishing after 1 hour. In contrast, MEK5-depleted cells displayed not only higher levels of phospho-CHK2 (Thr^{68}) at 15-30 min compared with control (siLuciferase) cells, but phosphorylation remained elevated for up to 8 h (**Figure 7**). Interestingly, a long term experiment revealed that phospho-CHK2 (Thr^{68}) levels were high even 4 days post-IR (**Figure 8**). Increased and persistent levels of phospho-CHK2 (Thr^{68}) were also detected in another human prostate cell line, PC3MM2 (**Figure 7**).

In contrast to CHK2 activation, CHK1 phosphorylation at Ser^{345} was significantly higher in the irradiated, control, DU145, PC3, or PC3MM2 cells, while MEK5 knockdown impaired CHK1 activation (**Figure 7**).

Finally, we induced elevated levels of phospho-CHK2 (Thr^{68}) in DU145 cells exposed to 4 Gy γ-rays transfected with an *ERK5* siRNA, as well as using a MEK5-specific inhibitor (BIX02189) (**Figure 9**).

Next, we examined the impact of MEK5 downregulation on the activation of ATM and ATR, which are apical kinases in the DDR pathway. As shown in **Figure 10**, MEK5 knockdown impairs activation and phosphorylation at Ser^{1981} of ATM in both DU145 (two independent experiments using two non-overlapping MEK5 siRNAs) and PC3 cells in response to IR. Activation of ATM was not only reduced in magnitude in MEK5 depleted cells, but it was also shorter in duration with phospho-ATM (Ser^{1981}) being detectable 24 h post-IR in MEK5-replete cells (**Figure 10**). SMC1 (structural maintenance of chromosome 1) protein, which is phosphorylated by ATM at Ser^{957} in response to genotoxic stress, followed the same pattern of activation in irradiated MEK5-control and MEK5-knockdown DU145 cells as ATM.

In contrast to ATM activation in response to IR, ATR activation, as determined by phosphorylation of Ser^{428}, was not appreciably affected by MEK5 downregulation in DU145 or PC3 cells, and neither were phospho-DNA-PKcs (Ser^{2056}) levels changed appreciably in response to MEK5 knockdown in irradiated PC3 cells (**Figure 10**).

Specific Aim 2

Task 1: Evaluate the contribution of Akt to MEK5-induced radioresistance by employing both an Akt-specific inhibitor and Akt specific siRNAs in MEK5-expressing cells and expressing an active Akt construct in prostate cancer cells with reduced MEK5 levels: Measure the effect on cell radioresistance by clonogenic survival. Cell lines to be used: DU145, PC3 [ATCC].

Akt is phosphorylated in response to DNA damage and downregulation or inhibition of Akt sensitizes cells to IR (25-27). Irradiation of serum-starved PC3 cells leads to Ser^{473} phosphorylation and activation of Akt. Phospho-Ser^{473} increased 15 min post-IR reaching its maximum at 30 min, and then returned to near basal levels by 3 h (**Figure 11**). When MEK5 levels were reduced by siRNA, however, phosphorylation levels of Ser^{473} at its maximal level (30 min) were only ~35% of that of irradiated control PC3 cells (**Figure 11**). We also assessed the impact of MEK5 downregulation on Akt activation in response to IR in DU145 cells. DU145 cells normally express low levels of phospho-Akt. However, when these cells were irradiated, phospho-Ser^{473} levels increased, albeit with slower kinetics than PC3 cells. Thus, Akt phosphorylation peaked at around 8 hr post-IR, while phospho-Akt levels were still detectable 24 h later. As in the case of PC3 cells, MEK5 silencing resulted in reduction in phospho-Ser^{473} abundance in response to 4 Gy γ-rays (**Figure 12**).

Aim 2 is still in progress.

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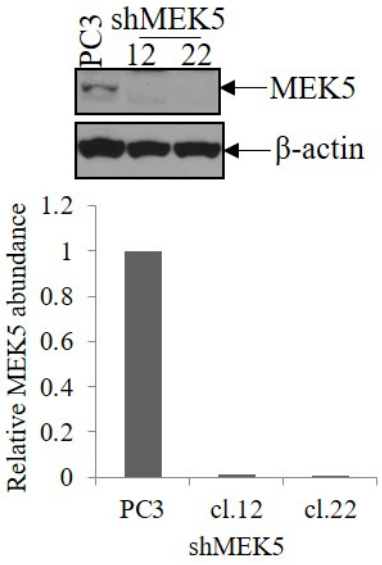

Figure 1: **PC3 clones stably expressing shMEK5**. PC3 cells were infected with lentiviral particles containing pLKO.1-shRNA against MEK5. Clones (12, 22) with shMEK5 knockdown were isolated after puromycin (1 μg/ml) selection.

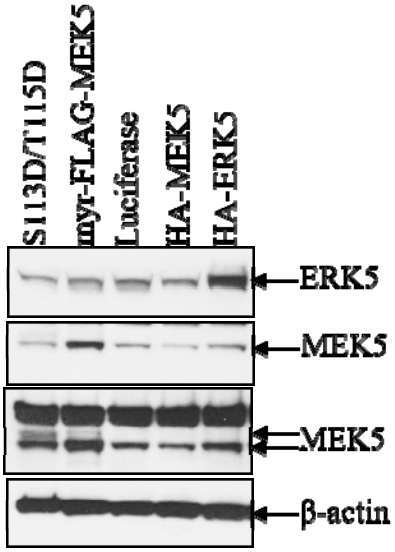

Figure 2: MEK5 constructs. HEK293T cells transiently transfected with 2 μg of the indicated plasmids for 27 h. S113D/T115D and myr-FLAG-MEK5: constitutively active MEK5; Luciferase: control; HA-tagged MEK5 and ERK5: wild type MEK5 and ERK5.

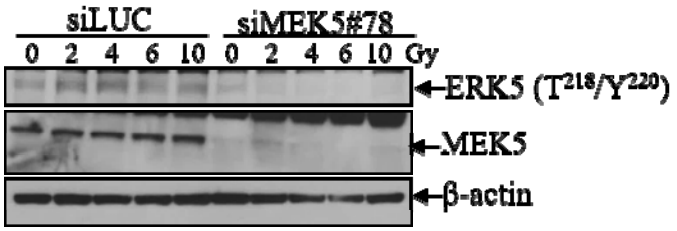

Figure 3. Dose response activation of ERK5 to ionizing radiation. DU145 cells were transiently transfected with siLUC or siMEK5 (#78). Two days later, cells were serum-starved for 24h and irradiated by various doses of γ-radiation. Twenty minutes later, cells were lysed and proteins were subjected to immunoblotting with the indicated antibodies.

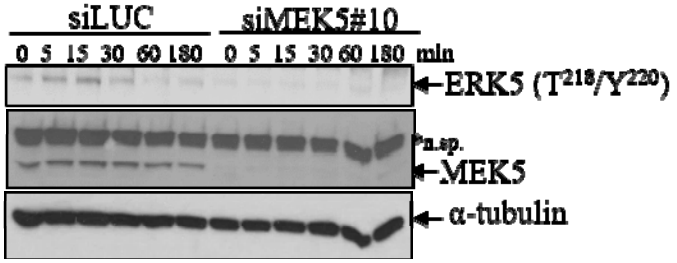

Figure 4: Time course activation of ERK5 in response to ionizing radiation. PC3 cells transiently transfected with either 50 nM Luciferase (siLUC) or MEK5 (siMEK5#10) siRNA were serum starved for 48h. Cells were irradiated with 4 Gy and lysed at the indicated time points. Levels of total MEK5 and α-tubulin are shown; * n. sp. , non-specific.

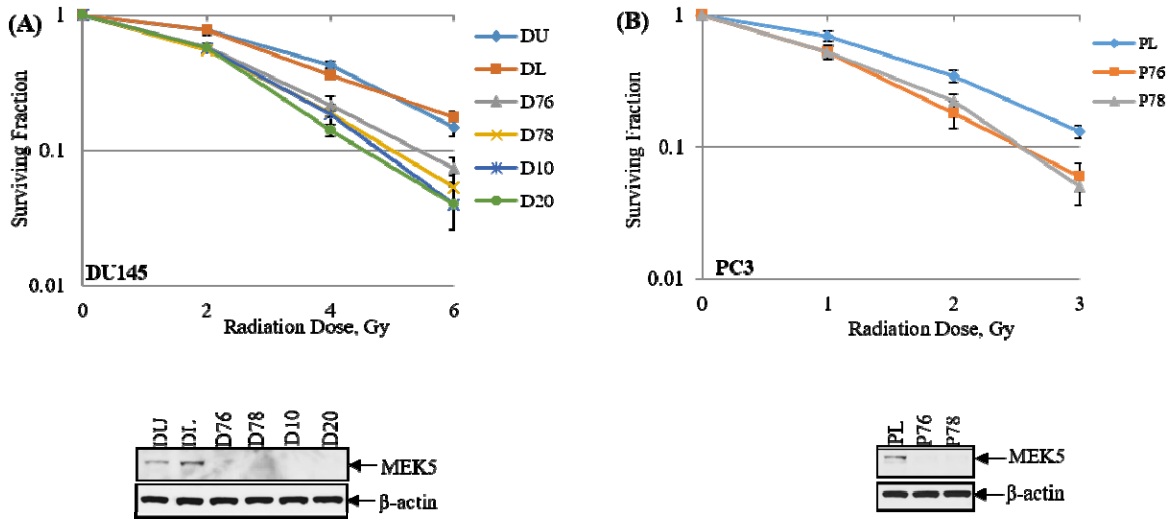

Figure 5: Clonogenic survival assay. (A), *Left panel*, DU145 cells were either left untransfected (DU) or transiently transfected with luciferase siRNA (DL) or four different siRNAs against MEK5 (D76, D78, D10, D20). Two days later cells were irradiated with increasing doses of γ-radiation and plated for clonogenic assay. After 11 days colonies were scored and normalized against plating efficiency. *Lower left panel*, western blot analysis showing MEK5 knockdown. **(B)** , *Right panel*, PC3 cells were transfected with luciferase siRNA (PL) as control or MEK5 siRNAs (P76, P78) and clonogenic assay was carried out as in (A). *Lower right panel*, western blot analysis showing MEK5 knockdown. Data represent the mean ± S.D from an experiment performed in triplicate.

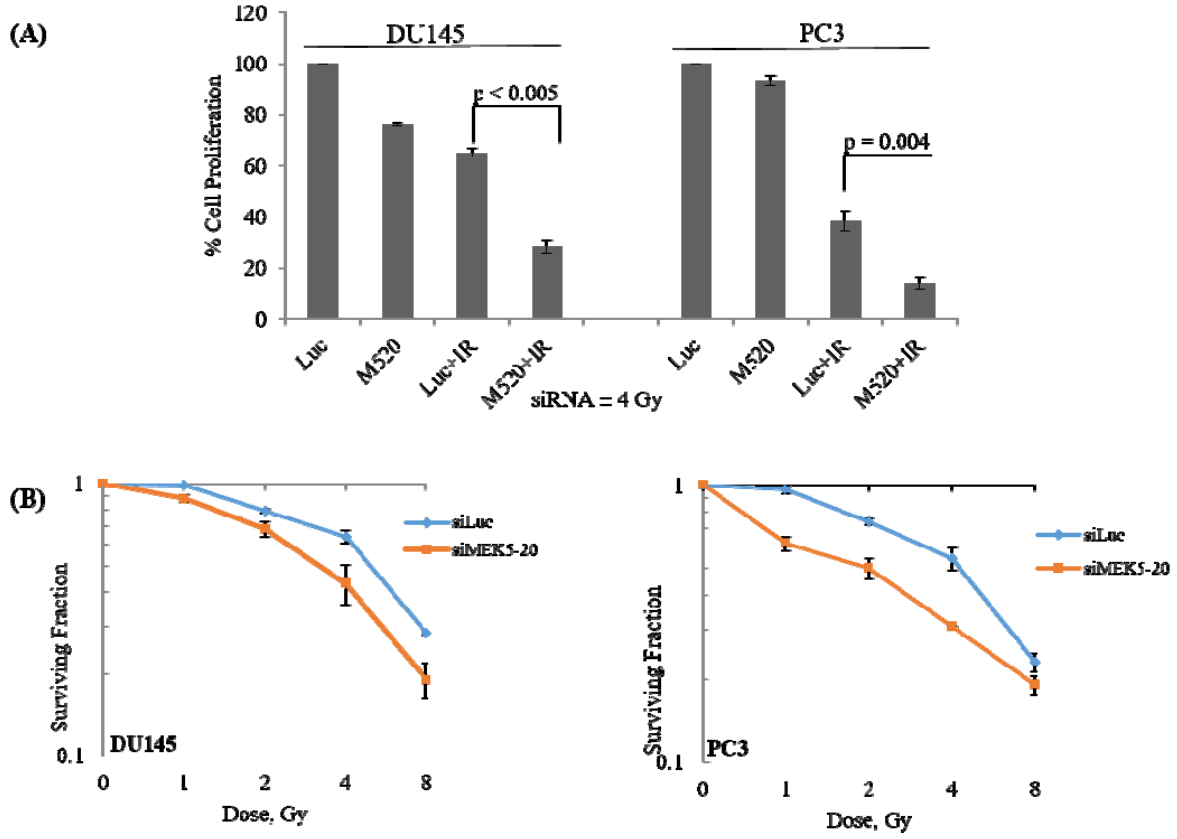

Figure 6: Cell proliferation assay. (A), DU145 and PC3 cells were transiently transfected with control luciferase (LUC) or MEK5 (M520) siRNA. Three day later, cells were irradiated with 4 Gy γ-rays and incubated for 6 days. Cells were trypsinized and counted with a hemocytometer. Shown mean ± S.D. (n= 3). **(B)**, DU145 and PC3 cells were transiently transfected with control luciferase (Luc) or MEK5 (M520) siRNA. Three day later, cells were irradiated with 1, 2, 4, or 8 Gy γ-rays and incubated for 6 days. Cells were fixed in 4% paraformaldehyde, stained with 0.5% crystal violet, and the stain was extracted with 10% acetic acid. Absorbance was measured at 590 nm and results were normalized relative to control siLuc cells. Shown mean ± S.D. (n= 3).

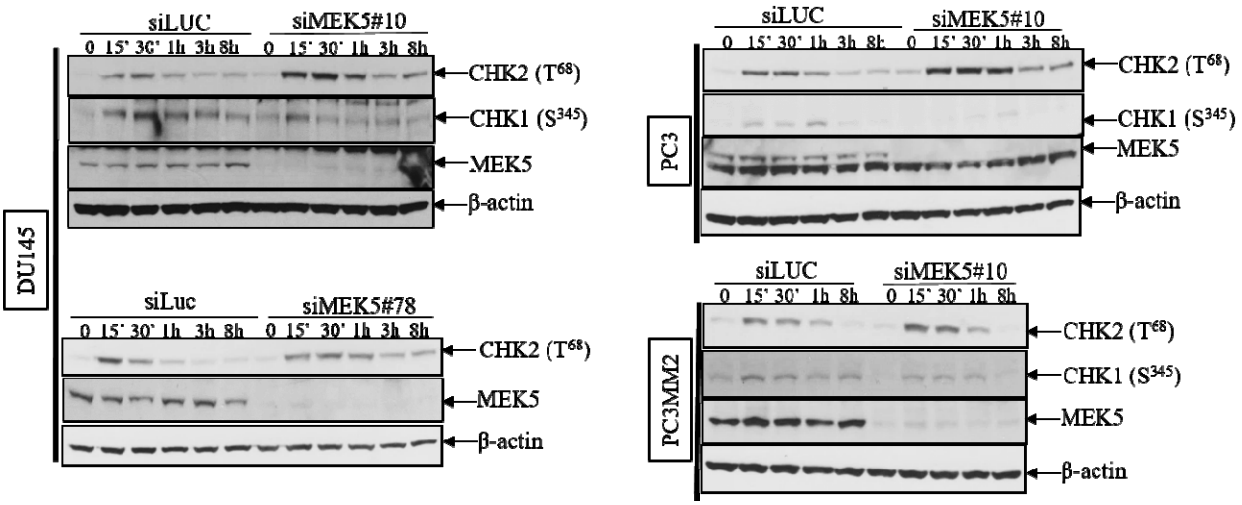

Figure 7: MEK5 impacts on CHK2 phosphorylation in response to IR. Prostate cancer cells were transiently transfected with 50 nM Luciferase or MEK5#10 siRNA or 25 nM MEK5#78 siRNA. Three days later, cells were serum starved for 24 hour (DU145) or 48 hr (PC3, PC3MM2) and irradiated with 4 Gy of γ-rays. Cells were lysed and immunoblotted with the indicated antibodies. Data for DU145 are from two independent experiments using either #20 or #78 siMEK5.

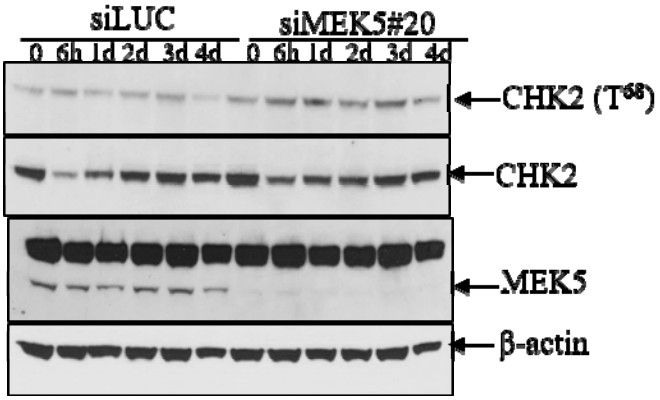

Figure 8: Long-term impact of MEK5 knockdown on phospho-CHK2 (T^{68}) in response to IR. DU145 cells transiently transfected with siMEK5 (#20) or siLuciferase (LUC) were exposed to 4 Gy γ-rays and lysates were collected at the indicated times for immunoblotting with phospho-CHK2, total CHK2, MEK5, and β-actin (loading control).

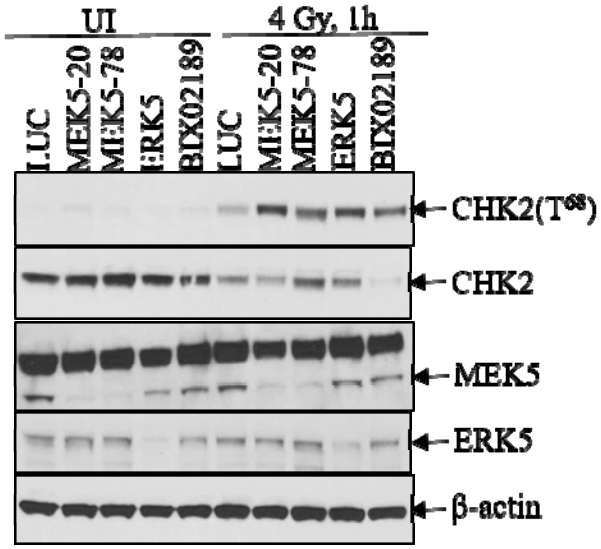

Figure 9: MEK5/ERK5 pathway inhibition results in increased phospho-CHK2 levels. DU145 cells transiently transfected with siLuc, siMEK5 (#20, #78), siERK5, or treated overnight with 10 μM BIX02189, were exposed to 4 Gy γ-rays or sham irradiated (UI). One hour later, cells were lysed and immunoblotted with the indicated antibodies.

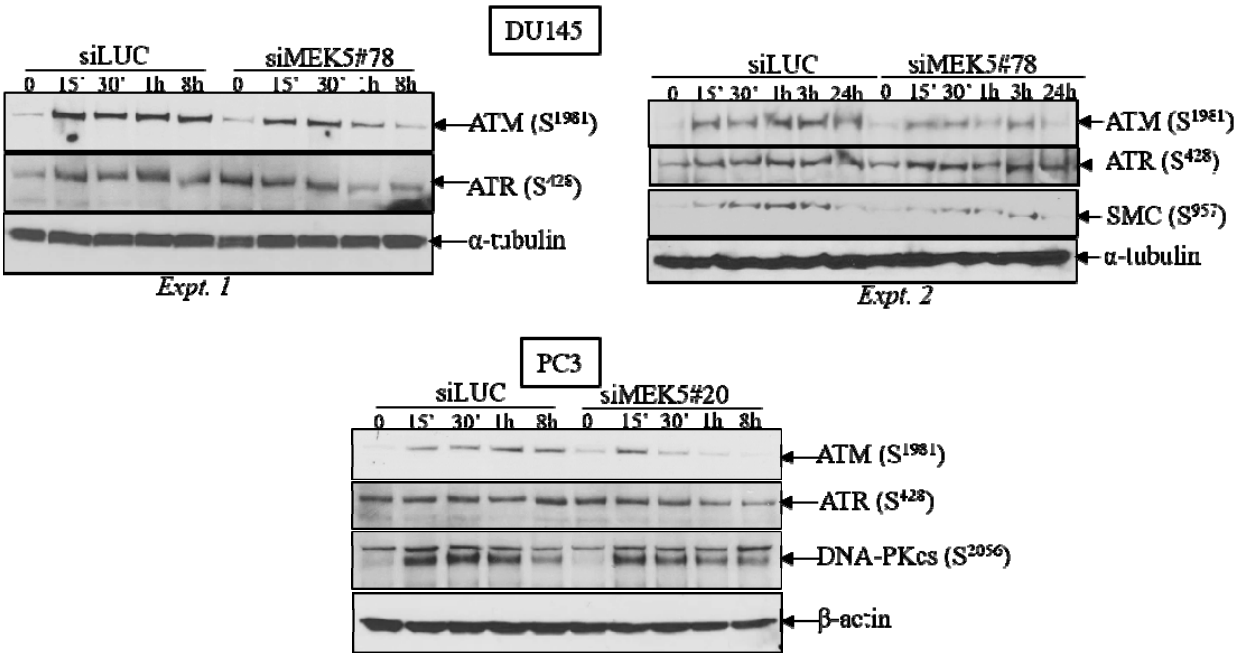

Figure 10: Effect of MEK5 silencing on ATM activation in response to IR. DU145 and PC3 cells were transiently transfected with 50 nM Luciferase or 25 nM MEK5#78 (DU145; two independent experiments) or 50 nM MEK5#20 (PC3) siRNA. Three days later, cells were serum starved for 24 hr and irradiated with 4 Gy of γ-rays. Cells were lysed and immunoblotted with the indicated antibodies.

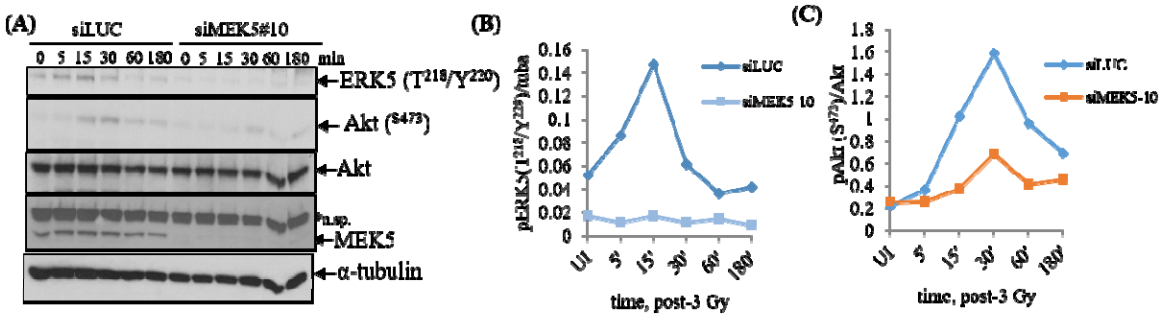

Figure 11: MEK5 is required for Akt activation after IR. (A) PC3 cells transiently transfected with either 50 nM siLuc or siMEK5#10 were serum starved for 48h. Cells were irradiated with 3 Gy and lysed at the indicated time points. Levels of phospho-Akt (S^{473}), total MEK5 and α-tubulin (loading control) were measured by immunoblotting. **(B)** Quantitation of phospho-ERK5 levels. **(C)** Quantitation of phospho-Akt levels. * n. sp., non-specific. Blot in (A) is a reprobe of the blot shown in Figure 4.

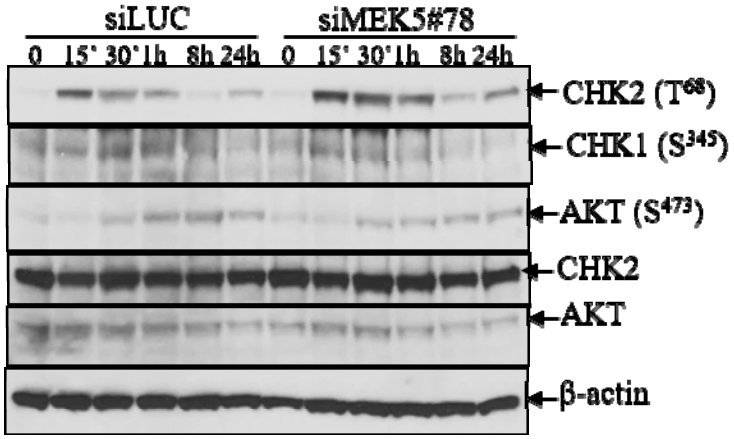

Figure 12: **MEK5 depletion impairs Akt activation in irradiated DU145 cells.** Cells were transiently transfected with siLUC or siMEK5 (#78). Four days later, cells were irradiated with 4 Gy γ-rays and lysed at the indicated times. Levels of phospho-CHK2 T^{68}), phospho-CHK1 (S^{345}), phospho-Akt (S^{473}), total CHK2, AKT, MEK5 and α-tubulin (loading control) were measured by immunoblotting.

What opportunities for training and professional development has the project provided?

Nothing to Report

How were the results disseminated to communities of interest?

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?

(1) Experiments are currently under way to determine the sensitivity of the immortalized and non-tumorigenic EP156T cells (28) to IR, as well as investigate the activation of ATM and CHK1/2 kinases. Subsequently, these cells will be infected with our lentiviral constructs expressing activated MEK5 and examine radioresistance.

(2) Immunofluorescence experiments are designed to determine γH2AX and 53BP1 foci formation. These experiments will be followed by examination of RAD51 foci formation, which is a marker of homologous recombination repair.

(3) We have already initiated experiments that will assess the impact of ATM and CHK2 inhibitors KU60019 and CHK2 inhibitor II, respectively, on DU145 and PC3 cell survival after irradiation. The inhibitor experiments will be supplemented with siRNA experiments targeting ATM and CHK2.

(4) We will quantify differences in mitotic index between control and MEK5 knocked down DU145 and PC3 cells by performing flow cytometric analysis of phospho-histone H3 positivity and DNA content.

(5) We will examine irradiated DU145 and PC3 cells with MEK5 knockdown for signs of abnormal mitoses (e.g. micronuclei formation, lagging DNA strands, etc) that would indicate mitotic catastrophe over a period of 5 days after irradiation.

(6) We plan to use an AKT-specific siRNA, as well as an AKT specific inhibitor (MK2206), and assess whether AKT depletion can phenocopy the effects of MEK5 silencing on ATM and CHK1/2 phosphorylation, as well as clonogenic survival using irradiated DU145 and PC3 cells.

(7) In the second half of the current funding period, we will examine the effect of MEK5 on irradiation-induced angiogenesis as outlined in the proposal.

(8) We will perform global gene expression using the newly isolated MEK5 shRNA clones from DU145 and PC3 cells.

IMPACT

What was the impact on the development of the principal discipline(s) of the project?

So far this study has yielded some significant findings that may have a major scientific impact on the field of radiation oncology. It is shown here that MEK5 downregulation affects major pathways related to the pathology of human prostate cancer, such as Akt signaling and DNA damage response and repair. Furthermore, MEK5 knockdown leads to reduced phosphorylation levels of ATM and its substrate, SMC; however it increases phosphorylation of another ATM effector, CHK2. This is a novel finding that has not been observed previously.

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

CHANGES/PROBLEMS

Changes in approach and reasons for change
No changes in approach are necessary

Actual or anticipated problems or delays and actions or plans to resolve them
None anticipated

Changes that had a significant impact on expenditures
Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Not used in this funding period

PRODUCTS

Publications, conference papers, and presentations

Poster Presentation
Broustas, C. G. Downregulation of MEK5 Sensitizes Human Prostate Cancer Cells to Ionizing Radiation. Abstract *in* PCRP - Innovative Minds in Prostate Cancer Today (IMPaCT), Towson, MD. August 4-5, 2016.

PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Constantinos G. Broustas
Project Role: PI
Nearest Person month worked: 5
Contribution to Project: Designed, performed, interpreted experiments

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report

What other organizations were involved as partners?

Nothing to Report

SPECIAL REPORTING REQUIREMENTS

Nothing to Report

APPENDICES

Abstract *in* PCRP - Innovative Minds in Prostate Cancer Today (IMPaCT), Towson, MD. August 4-5, 2016.

AUTHOR

Constantinos G. Broustas

Center for Radiological Research, Columbia University College of Physicians and Surgeons, New York, NY 10032

TITLE

Downregulation of MEK5 Sensitizes Human Prostate Cancer Cells to Ionizing Radiation

ABSTRACT

Background & Objectives: Tumor cell resistance to ionizing radiation (IR) poses a major obstacle in prostate cancer therapy. Mitogen/extracellular signal-regulated kinase kinase-5 (MEK5) belongs to the family of MAP kinases. It is activated by the upstream kinases MEKK2 and MEKK3. MEK5, in turn, phosphorylates and activates extracellular signal-regulated kinase 5 (ERK5) at Thr218/Tyr220. MEK5/ERK5 pathway plays a pivotal role in tumor initiation and progression, including prostate cancer. MEK5 protein is overexpressed in prostate cancer cells compared with normal cells and MEK5 levels are correlated with prostate cancer metastasis. This study explores the hypothesis that MEK5 is a contributing factor to the response of prostate cancer cells to IR and seeks to elucidate the mechanism by which MEK5 affects radioresistance.
Methods: Castration-resistant DU145, PC3, and PC3MM2, as well as androgen-dependent LNCaP prostate cancer cells were treated with MEK5 short interfering (si) RNA alone or in combination with γ-rays. Clonogenic survival assays, cell cycle analysis, immunofluorescence and immunoblotting were performed to assess cell proliferation, survival, cell cycle progression and DNA damage response.
Results: We examined MEK5/ERK5 pathway activation in response to IR in prostate cancer cells transiently expressing *Luciferase* (control) or *MEK5* siRNAs. Control cells with normal levels of MEK5 showed an increase in phospho-ERK5 levels at 5 and 15 min post-IR, diminishing at later time points. In addition, we discovered that AKT activation after 4 Gy IR was dependent on the presence of MEK5. AKT phosphorylation at Ser473, which is considered a marker of AKT activation, was increased reaching maximal levels at 30 min post-IR. In contrast, when MEK5 was downregulated by MEK5 specific siRNAs, AKT activation was severely impaired. Moreover, MEK5 silencing had an impact on the DNA damage response pathway. Specifically, MEK5 knockdown, combined with IR, resulted in significantly higher phospho-CHK2 (Thr68) levels 30 min after irradiation compared with irradiated cells with endogenous levels of MEK5. Additionally, increased levels of phospho-CHK2 persisted for at least 8 h post-irradiation, whereas the phospho-CHK2 signal returned to near basal levels by 3 h in control cells. On the other hand, CHK1 phosphorylation at Ser345 and activation in response to IR was elevated in MEK5 control cells 30 min post-irradiation compared with MEK5 knockdown cells. Finally, MEK5 depletion by two non-overlapping siRNAs sensitized prostate cancer cells to IR as determined by clonogenic survival assay. Short-term targeting of MEK5 in combination with IR led to approximately 70% reduction in prostate cancer cell proliferation 6 days post-irradiation.

Conclusions: These data indicate that MEK5 knockdown radiosensitize prostate cancer cells. In response to IR, MEK5 controls activation of AKT, a kinase involved in radioresistance, as well as DNA damage response by regulating activation of CHK1/2 kinases. Ongoing studies focus on determining the contribution of AKT and CHK1/2 kinases and their downstream effectors to MEK5-dependent radioresistance.

Impact: This study focuses on mechanisms of resistance to radiotherapy for patients with localized prostate cancer. Downregulation of MEK5 can selectively radiosensitize prostate tumors, while sparing normal tissue, thus improving survival of cancer patients.